A STUDY OF CHROMOSOMES OF TOXOPNEUSTES VARIEGATUS WHICH SHOW INDIVIDUAL PECULIARITIES OF FORM.

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INTRODUCTION.

The observations described in this paper were made during the winter and spring, 1909–10, in the Biological Laboratory of Bryn Mawr College.[1] Last November after my arrival in Bryn Mawr Professor Tennent suggested to me a study of the chromosomes in eggs of certain echinoderms with special reference to characteristic peculiarities of form, a question especially significant since the appearance of Baltzer's ('09*a*) paper on the chromosomes of *Strongylocentrotus lividus* and *Echinus microtuberculatus* which has thrown new light on the individuality of chromosomes in echinoids. Previous to this author's first publication on the subject ('08) there existed only a suggestion by Boveri ('01 and '07) that in some echinoids there occur chromosomes of a characteristic shape. Baltzer pointed out that in *Echinus microtuberculatus* there are two very long rod-shaped chromosomes, two long hook-shaped ones, and two or three horseshoe-shaped chromosomes, while in *Strongylocentrotus lividus* there are two long rod-shaped chromosomes, two long hook-shaped ones and in a part of the eggs one smaller hook-shaped chromosome. As this latter one occurs only in a part of the eggs—in about one half of them—Baltzer suggests ('09*a*, p. 596) that it is probably an idiochromosome whose smaller mate is one of the shorter rod-shaped chromosomes. The same suggestion is made in regard to one of the horseshoe-shaped chromosomes in *Echinus*, in cases where there are three of that type. While idiochromosomes and other heterochromosomes have for some time been known in insects, arachnids and myriapods,

[1]I take advantage of this opportunity to express my sincere thanks for the generous scholarship awarded to me by Bryn Mawr College and for encouraging suggestions from Professor Tennent and Dr. Stevens during the course of my work in their laboratory.

they have only quite recently been discovered by Gulick in the nematode *Heterakis* (Boveri, '10), by Baltzer in the echinoids, and by Guyer ('09*a* and *b*) in vertebrates.

Observations on metakinesis stages (Baltzer '09*a*, Plate XXXVII., Fig. 9) have shown that the hook-shape of certain chromosomes in *Strongylocentrotus* is due to the fact that the spindle fiber from each pole is attached at a point about one third of the length of the chromosome from one end, so that a shorter and a longer arm are formed. In the horseshoe-shaped chromosomes in *Echinus* the fiber is attached about half way between the two ends, so that the two arms are nearly of the same length (*ibid.*, Fig. 10). As for the origin of the hook-shaped and horseshoe-shaped heterochromosomes, observations on cross-fertilized eggs, $\frac{\textit{Strongylocentrotus}\ ♂}{\textit{Echinus}\ ♀}$, and on multipolar mitoses of *Strongylocentrotus* have shown that they come from the female pronucleus, the corresponding pair in the male being rod-shaped. Apparently the female has an unequal pair of heterochromosomes, one hook- or horseshoe-shaped, the other rod-shaped; while the male has a corresponding equal pair of rod-shaped chromosomes. It may be mentioned that this is the reverse of what is found in insects, but as in most insects the female nucleus must obtain more chromatin than the male nucleus.

Material and Methods.

My observations were made upon eggs of *Toxopneustes* and *Arbacia*, collected and preserved by Professor Tennent. As preserving fluid either picro-acetic or sublimate-acetic was used. Sections of 5 μ thickness were stained with Heidenhain's iron hæmatoxylin, except in a few cases mentioned later.

The figures are all drawn with Abbe's drawing camera, Zeiss oil immersion 2 mm. apochr. objective, oc. 12, enlarged to twice or four times the original diameter and reduced one half.

Arbacia punctulata.

The eggs of this species are quite unfavorable for detailed cytological studies. Not only are the chromosomes very small but the cytoplasm of the egg is filled with pigment granules so

that a sharp differentiation of plasma and chromosomes is impossible. Following Dr. Stevens's suggestion, I tried to bleach the section with H_2O_2, a method successfully applied in some other cases, but entirely useless in *Arbacia*. I therefore gave up further study of the chromosomes of this species.

Toxopneustes variegatus.

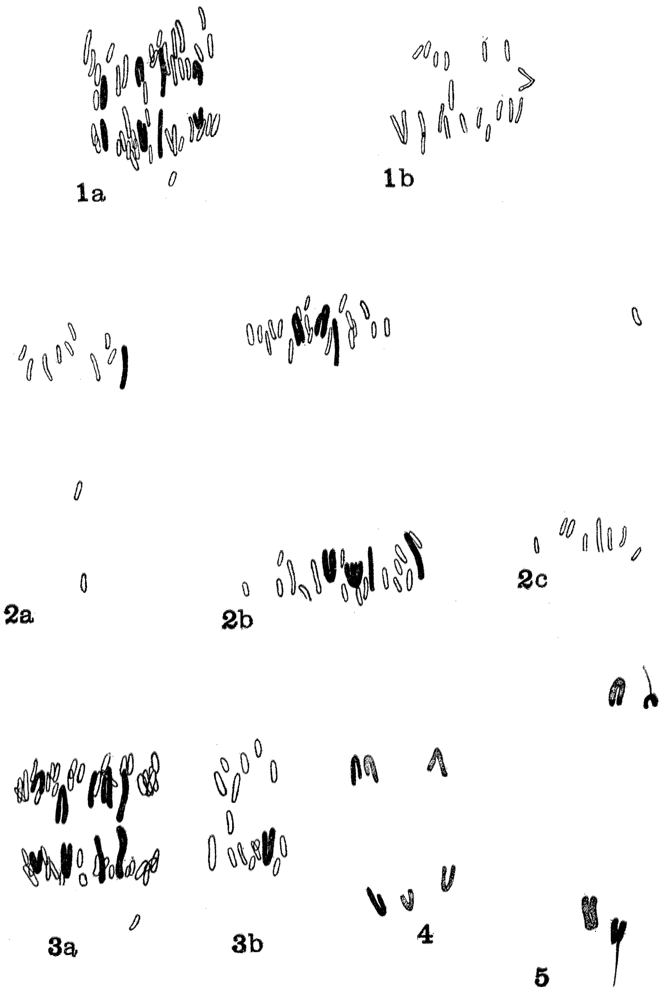
The observations were made on two series of eggs from two different localities, one from Beaufort, N. C., the other from the Tortugas. The results obtained are the same for both. I began with the study of the chromosomes in the first segmentation spindle and found in *Toxopneustes* as Baltzer ('09*a*) had done in *Strongylocentrotus* and *Echinus* a considerable variation in the length and form of the chromosomes.

As in *Echinus* and *Strongylocentrotus* there are two extremely long chromosomes in each daughter plate. Their behavior resembles that described by Baltzer in that these long rod-shaped chromosomes are often late in splitting and moving to the poles (compare Text-fig. 3, *a*, with Baltzer's '09*a*, Pl. XXXVII., Fig. 5, *a* and *b*). These chromosomes may also be seen in my Figs. 1, 2 and 3. Sometimes these long chromosomes are contracted more and then appear thicker and shorter (Fig. 1, *a*, the left pair of long rod-shaped chromosomes).

A type of chromosome of peculiar shape, found in all of the eggs, is one which is usually V-shaped, but sometimes more horseshoe-shaped (Figs. 4 and 5). Very frequently in late anaphases the two arms are parallel, or nearly so, and one may partly overlie the other, but there is hardly ever any doubt as to whether there is one chromosome with two arms present or two separate rod-shaped ones (Fig. 3, *a* and *b*). As may be observed from Fig. 4 the length of the two arms in these chromosomes may vary slightly when the arms are lying in one optical plane. I tried to determine whether this difference is confined only to certain chromosomes or to chromosomes in certain eggs, but no regularity seems to exist. The probable origin of the difference in length will be discussed later.

These V-shaped chromosomes occur in all fertilized *Toxopneustes* eggs and there are either two or three present. Among 34

one-cell stages examined with reference to this point, I found in 16 cases two, and in 18 three such chromosomes. Fig. 1, *a*, illustrates a case with two V-shaped chromosomes in each daughter plate. Their antagonistic position proves that they are division



FIGS. 1–5.

products of the same chromosome, their regular number, that they are not merely incidental features. The other chromosomes are very crowded, as most of them occur in one section. Their position has been slightly changed in cases where they were

over or under the V-shaped chromosomes, and this holds for all similar figures. Special care was of course taken to keep the position of the long rod-shaped and the V-shaped chromosomes in every case as accurately as possible.

Fig. 2, *b*, shows daughter plates of a late anaphase where there are three V-shaped chromosomes with more nearly parallel arms. Two of these chromosomes are very close together, one partly overlying another in each plate. Fig. 3 also shows in *a* and *b* three V-shaped chromosomes in early anaphase. One pair of these chromosomes appears smaller than the two others, but in examining other cases I found no regularity in the apparent size of the three pairs. Sometimes all three pairs vary a little, sometimes they are all of apparently the same size, sometimes only the members of one pair vary in apparent size. This difference may be due to difference in contraction or to an original difference in length of the chromosomes. As Baltzer's ('09*a*, p. 568) measurements of the hook-shaped chromosomes show, the length of chomosomes of a certain type is quite variable; it may vary from 9.75–14.0 mm. There is no resemblance however to the conditions in *Strongylocentrotus* in respect to the third pair of hook-shaped chromosomes which always is considerably smaller than the other two.

One of the three V-shaped chromosomes is probably a heterochromosome, as Baltzer assumes to account for the conditions found in *Strongylocentrotus* and *Echinus*. Studies in ovogenesis and spermatogenesis would be necessary to obtain evidence for or against the suggestion made.

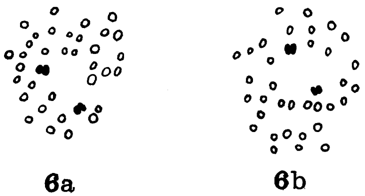
As it is difficult to count the total number of chromosomes in a lateral view of the daughter plates I counted the number in polar views where fortunately the V-shaped chromosomes show very clearly.

Fig. 6, *a* and *b*, represents two succeeding sections through two anaphase daughter plates; the V-shaped chromosomes are the two double ones, finished in solid black. The number of chromosomes in each plate is 36. Fig. 7 shows a polar view with three V-shaped chromosomes; here also the number is 36.

I counted 14 anaphase plates from the pole, with a clear arrangement of chromosomes, and found the average number

36, always counting the two arms of the V-shaped chromosome as one.

Although I did not have very much material I was nevertheless able to trace the V-shaped chromosomes in the 2-, 4-, 8-, 12- and 16- to 32-cell stages.



6a 6b

FIG. 6.

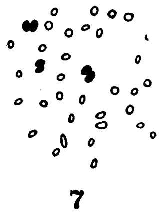


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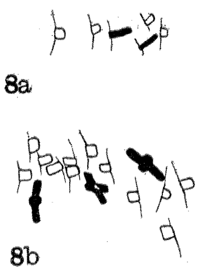
FIG. 7.

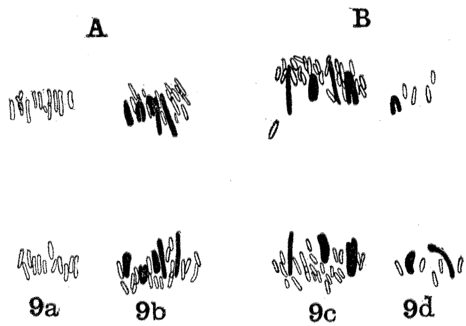
Fig. 8, *a* and *b*, shows a metaphase from a 2-cell stage, with the three V-shaped chromosomes distinguishable by their characteristic splitting figures, which are fully explained below.

Fig. 9, *A* and *B*, represents two adjoining cells of a 16- to 32-cell stage. In cell *A*, 9*b*, we see three V-shaped chromosomes



8a 8b

FIG. 8.



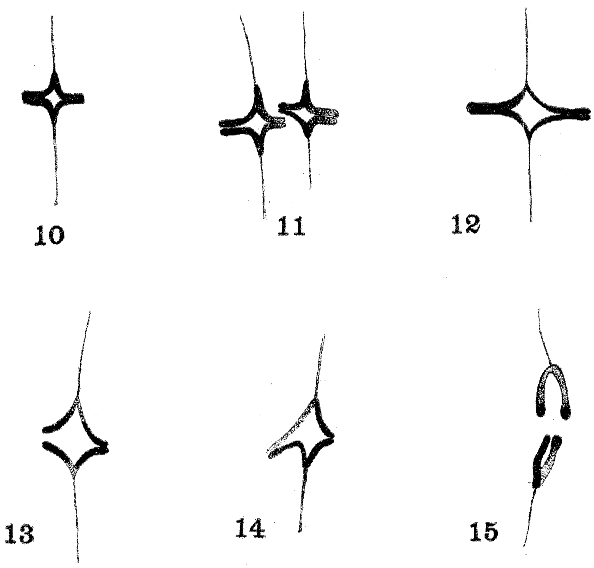
A B 9a 9b 9c 9d

FIG. 9.

and the two long ones. In *B*, 9*c*, we see again two of the V-shaped chromosomes; and in 9*d* the third one. The two long ones are distributed between *c* and *d*. The chromosomes in this stage are so small and crowded together that an accurate count of their total number is impossible.

The figures 10–15, enlarged 4 diameters and in publication reduced one half, show splitting, or metakinesis, stages of the

V-shaped chromosomes. From these figures it is evident that the V-shaped chromosomes of the anaphase come from a long rod-shaped chromosome to which the spindle fibers are attached with more or less regularity in the middle of the chromosome (Fig. 12). If the separation of the daughter-chromosomes takes place earlier at one end than at the other (Fig. 13) or one arm of the chromosome is contracted more than the other, or, as Fig. 14 suggests, if some hindrance on one side prevents one



FIGS. 10–15.

end from moving toward the pole as rapidly as the other end, then the variations in the length of the arms mentioned above (Fig. 4) appear. In comparing these figures with Baltzer's '09*a*, Pl. XXXVII., Fig. 10, we cannot fail to find a close resemblance.

In Baltzer's paper '09 *a*, p. 607, we notice a suggestion that in the hook-shaped chromosomes there may be a union of two chromosomes, end to end at the point where spindle fibers are attached. The reasons given for this suggestion are the extraordinary length of the hook-shaped chromosomes, and the fact that all other chromosomes are attached to the spindle fibers by one end. This suggestion is a very natural one, for such apparently homogeneous but plurivalent chromosomes are

known in *Ascaris*, and compound chromosomes are found in the maturation mitoses of certain insects (McClung, '05, Payne, '09). In some of these latter cases (Payne, '09) the plurivalent chromosome has a spindle fiber attached to each unit in the early metaphase and in many cases two spindle fibers from each pole are attached to one of each of the four units of a tetrad in primary maturation mitoses (Stevens, '10).

Since the V-shaped chromosome of *Toxopneustes* seems to be exactly comparable to the hook-shaped chromosome of *Echinus* and *Strongylocentrotus*, the question arose whether, assuming that the V-shaped chromosomes of *Toxopneustes* may be bivalent, one might by careful observation be able to trace in the early metaphase two spindle fibers from each pole attached to each of them. As the spindle fibers were not especially clear in the preparation stained with iron-hæmatoxylin alone, a few slides were counter-stained with Rubin S. Among 42 cases I found only two where I was inclined to count two fibers; in all other cases I was certain that only one fiber from each pole was attached to each V-shaped chromosome. My observations have therefore failed to add any facts supporting Baltzer's suggestion, which, however, future investigation may verify.

Baltzer ('09*a*) traced the heterochromosome in *Strongylocentrotus* and *Echinus* to the female pronucleus. Unfortunately I was not able to obtain suitable material for this purpose, but further investigation will probably reveal the same conditions as in *Echinus* and *Strongylocentrotus*.

Discussion.

Comparing the chromosomes with peculiar shape in *Echinus* and *Strongylocentrotus* with those in *Toxopneustes* we find that the hook-shaped chromosomes in *Echinus* and *Strongylocentrotus* have no exact equivalent in *Toxopneustes*. The two extremely long rod-shaped ones are found in the three species. The V-shaped chromosomes in *Toxopneustes* are very similar to the horseshoe-shaped chromosomes of *Echinus* in respect to their formation and the equal length of their two arms. They differ from the *Echinus* chromosomes as already mentioned in their length and slenderness. As in *Echinus* we are not able to

distinguish a particular one of these three V-shaped chromosomes as a heterochromosome.

The discovery of individuality of form among the chromosomes in echinoids is a very valuable factor in support of Boveri's "Individualitätstheorie" of the chromosomes. One also welcomes every such means of distinguishing parental chromosomes in cross-fertilized eggs. *Toxopneustes* for instance has been used for cross-fertilization (Tennent, '07 and '10). These chromosomes which show marked individuality of form will be of special value in cases of cross-fertilized eggs, where, as shown by Herbst ('09) and Baltzer ('09*b*), the chromosomes of one parent are almost entirely eliminated during the first segmentation divisions.

LITERATURE.

Baltzer, F.

'08 Über die Grösse und Form der Chromosomen bei Seeigeleiern. Verh. d, d. Zool. Ges., 1908.

'09a Die Chromosomen v. Strongyl. liv. u. Ech. micr. Arch. f. Zellforsch., Bd. II.

'09b Über d. Entwicklung d. Echiniden-Bastarde mit bes. Berücksichtigung d. Chromatinverhältnisse. Zool. Anz., Bd. XXXV.

Boveri, Th.

'01 Zellenstudien. Heft IV. Jena.

'07 Zellenstudien. Heft VI. Jena.

'10 Über Geschlechtschromosomen bei Nematoden. Arch. f. Zellforsch.. Bd. IV.

Guyer, M. F.

'09a The Spermatogenesis of the Domestic Guinea (Numida meleagris dom.). Anat. Anz., Bd. XXXIV.

'09b The Spermatogenesis of the Domestic Chicken (Gallus gallus dom.). Anat. Anz., Bd. XXXIV.

Herbst, C.

'09 Vererbungsstudien, VI. Arch. f. Entw. Mech., Bd. XXVII., Heft 2.

McClung, C. E.

'05 The Chromosome Complex of Orthopteran Spermatocytes. Biol. Bull., Vol. IX.

Payne, F.

'09 Some New Types of Chromosome Distribution and Their Relation to Sex. Biol. Bull., XVI.

Stevens, N. M.

'10 An Unequal Pair of Heterochromosomes in Forficula. Journ. of Exp. Zoöl., vol. 8.

Tennent, D. H.

'07 The Chromosomes in Cross-fertilized Echinoid Eggs. Biol. Bull., XV.

'10 The Dominance of Maternal and of Paternal Characters in Echinoderm Hybrids. Arch. f. Entw. Mech., Bd. XXIX.